

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Field Crops Research 94 (2005) 14–32

**Field
Crops
Research**www.elsevier.com/locate/fcr

Field evaluation of drought tolerance QTL effects on phenotype and adaptation in pearl millet [*Pennisetum glaucum* (L.) R. Br.] topcross hybrids

F.R. Bidinger^{a,*}, R. Serraj^a, S.M.H. Rizvi^a, C. Howarth^b, R.S. Yadav^b, C.T. Hash^a^aInternational Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru PO 502 324, Andhra Pradesh, India^bInstitute of Grassland and Environmental Research, Aberystwyth SY23 3EB, UK

Received 18 February 2004; received in revised form 8 November 2004; accepted 16 November 2004

Abstract

Marker-assisted breeding could significantly increase progress in improving crop drought tolerance, if QTL with significant effects on crop yield in stress environments can be identified. The objective of this research was to obtain a first assessment of a putative drought tolerance QTL on linkage group 2 (LG 2) of pearl millet. This was done by comparing hybrids made with topcross pollinators (TCP) based on progenies selected from the original mapping population for presence of the tolerant allele at the target QTL versus field performance in the phenotyping environments. A set of 36 topcross hybrids was evaluated in 21 field environments, which included both non-stressed and drought-stressed treatments during the flowering and grain filling stages. The QTL-based hybrids were significantly, but modestly, higher yielding in a series of both absolute and partial terminal stress environments. However, this gain under stress was achieved at the cost of a lower yield in the non-stressed evaluation environments. This particular pattern of adaptation in the QTL-based hybrids was consistent with their general phenotype—early flowering, limited effective basal tillering, low biomass and a high harvest index (HI)—which resembled that of the drought tolerant parent of the original mapping population. The results thus confirmed the effectiveness of the putative drought tolerance QTL on LG 2, but suggested that it may enhance drought tolerance by favoring a particular phenotype with adaptation to terminal stress.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Drought tolerance; Quantitative trait loci; Pearl millet; Phenotype

1. Introduction

Marker-assisted plant breeding (MAB) potentially offers significant gains in efficiency in crop improvement (Stuber et al., 1999). Expectations from this approach are particularly high in the case of complex traits or responses to complex environmental chal-

Abbreviations: DRI, drought response index; HI, harvest index; LG, linkage group; MAB, marker-assisted breeding; PNHI, panicle harvest index; QTL, quantitative trait loci; TCH, topcross hybrid; TCP, topcross pollinator

* Corresponding author. Fax: +91 40 232 41 239.

E-mail address: f.bidinger@cgiar.org (F.R. Bidinger).

lenges. Such responses are frequently: (1) conditioned by a range of individual component traits whose individual effects are very difficult to discern at the phenotype level (Nguyen et al., 1997; Prioul et al., 1997); and/or (2) normally subject to large genotype \times environment interaction. MAB may thus be particularly relevant to the genetic improvement of drought tolerance. MAB provides opportunities for both the introgression of various individual physiological or biochemical tolerance traits (e.g. Ludlow and Muchow, 1990; Turner et al., 2000) and/or for selection for complex, whole crop responses involved in crop tolerance to water deficits. Systematically pyramiding tolerance traits, which individually may have only a limited effect on the overall phenotype, in selected genotypes can provide a significant cumulative effect on crop yield under stress (Quarrie et al., 1999; Ribaut and Betran, 1999; Schneider et al., 1997; Nguyen et al., 1997). Similarly MAB can be effective in a yield-based, as well as a trait-based, approach to crop improvement for stress environments, as it allows the incorporation of QTL for superior expression of major yield components (e.g. grain number, grain filling, harvest index (HI)) under stress, where there are regular associations between such components and overall grain yield (Richards, 1996). QTL that account for a reasonable proportion of the variation in component expression or $G \times E$ variation can be used similarly to markers for simple traits in a MAB program, even though the responses they represent are “black boxes” in terms of our understanding of the underlying mechanisms involved (Quarrie et al., 1999; Schneider et al., 1997).

ICRISAT is investing considerable effort in evaluating MAB opportunities in improving tolerance to terminal (unrelieved, end-of-season) drought stress in pearl millet hybrid parents. This particular type of stress is common in arid and semi-arid pearl millet growing areas (van Oosterom et al., 1996; Eldin, 1993) and results in major yield losses (Mahalakshmi et al., 1987). Pearl millet is well adapted to a MAB approach because of its relatively small effective genome size (i.e. short linkage map length despite moderately large DNA content), high degree of genetic and molecular polymorphism (Breese et al., 2002), and the availability of easy-to-use PCR-based markers including single sequence repeats (SSRs) (Allouis et al., 2001; Budak et al., 2003; Qi et al., 2001, 2004). The work is focused on hybrids, for India, because an estimated

50% coverage of area sown to millet by F_1 hybrids and the existence of an efficient seed industry will guarantee the rapid adoption of more drought-tolerant hybrids. First attempts concentrated on identifying markers for the ability to maintain both grain yield, key yield components, and overall partitioning to grain yield (harvest index) under terminal stress. The work is based on populations created from elite hybrid parental lines (Yadav et al., 2002, 2004).

The research reported in this paper is the first attempt to assess the value of a major QTL identified on linkage group 2 (LG 2) for a positive expression of both grain yield and harvest index under terminal stress (Yadav et al., 2002). Because the original mapping population was based on two elite restorer lines, it contained a high frequency of desirable lines on which there are extensive molecular and field data from the genotyping and phenotyping of the mapping population. We exploited these data to create three topcross pollinators (TCP), or restricted restorer populations, by random mating sets of selected mapping population progenies based on their genotypic and phenotypic evaluation data. The first TCP (QTL TCP) was made from progenies homozygous for marker alleles from donor PRLT 2/89-33 flanking the putative quantitative trait locus on LG 2, which was associated with superior grain yield and HI under terminal stress (Yadav et al., 2002). It was compared to TCPs made from an equal number of progenies from the same mapping population that were selected on the basis of a high grain yield under stress in the field phenotyping evaluations (Field TCP). Also included in the experiment was a control TCP, which was made from progenies selected at random, to represent the general performance level of the population (Random TCP). The objectives of the research were: (1) to compare the performance of the marker-based and field-based TCH under both stress and non-stress environments; and (2) to assess the effects of the putative drought tolerance QTL on LG 2 on the phenotype and the adaptation of TCHs carrying it.

2. Materials and methods

2.1. Genetic materials

The TCPs used in this research were bred from mapped F_2 -derived F_4 lines from a population bred

from the cross of restorer lines H 77/833-2 and PRLT 2/89-33. H77/833-2 is a drought sensitive, high tillering line with small panicles, derived from north Indian landrace germplasm and the parent of several widely grown commercial hybrids (Kapoor et al., 1989). PRLT 2/89-33 is a drought tolerant, low tillering, large panicle experimental line derived from the widely adapted *Iniadi* germplasm from West Africa (Andrews and Anand Kumar, 1996). These two parents differed only moderately in flowering photo-period-temperature response. This made it easier to identify QTL associated with drought tolerance per se, instead of drought escape due to early flowering (which can be manipulated effectively in conventional breeding programs), with a modest sized testcross mapping population. The breeding, genotyping and phenotyping of this mapping population have been described (Yadav et al., 2002). Putative QTL were identified for grain yield and several of its key components under terminal drought stress. One of these QTL, on LG 2, explained as much as 23% of the phenotypic variation for maintenance of normal (non-stressed) grain yield under terminal stress (Yadav et al., 2002).

Eighteen F₄ progenies in each of three categories were selected from the mapping population based on the following criteria:

- *QTL TCP*: progenies homozygous for PRLT 2/89-33 alleles at RFLP marker loci *Xpsm25*, *Xpsm321*, *Xpsm592* and *Xpsm443* in the vicinity of the major drought QTL detected on LG 2 (see Yadav et al., 2002, for the location of the QTL on LG 2).
- *Field TCP*: progenies with the best overall ability to maintain non-stressed grain yield across all the terminal stress field phenotyping environments, irrespective of the presence or absence of favorable alleles at the putative drought tolerance QTL.
- *Random TCP*: randomly selected progenies from the entire mapping population, irrespective of the presence or absence of favorable alleles at the putative drought tolerance QTL, or of performance in the terminal stress phenotyping environments.

All 18 progenies in each group were testcrossed to a common A₁ cytoplasm male-sterile seed parent (A-line) and their restoration ability evaluated in the F₁ generation by covering 8–10 panicles with selfing

bags and scoring seed set at maturity. Based on the restoration ability, general plant appearance and uniformity of their testcrosses, 12 progenies were selected in each TCP type. These were recombined during the following season by hand pollinating a similar number of panicles from each progeny with bulk pollen from all 12 progenies. Equal amounts of crossed seed from each progeny within a group were bulked to form the three TCPs. Each TCP was then crossed to a common set of 12 A-lines to make 12 TCHs representing each selection criterion. The A-lines were divided equally into those producing hybrid phenotypes suitable for: (1) the arid zone of NW India—early maturity, high tillering, small panicle and adaptation to variable moisture environments (5054A, 841A, 843A, 88004A, 89111A, and ICMA 94444) and (2) the more favorable semi-arid areas of north central and peninsular India—lower tillering, large panicle and seed size and a high yield potential (ICMA 92777, ICMA 97111, ICMA 97555, ICMA 98333, ICMA 99111 and ICMA 99222). It is not known if any of the A-lines also carry the favorable (PRLT2/89-33) allele at the putative drought tolerance QTL on LG 2. PRLT2/89-33 was bred from the widely used *Iniadi* landrace germplasm (Andrews and Anand Kumar, 1996); 10 of the A-lines have varying amounts (15–100%) of *Iniadi* germplasm in their pedigrees. Therefore, the chances of some of the A-lines containing the favorable allele are high.

2.2. Field evaluation environments

2.2.1. Non-stress environments

The 36 TCHs were evaluated in replicated trials in a range of non-stress and terminal stress environments. These included trials in both the normal rainy season, and in the rain-free dry season in which the original phenotyping for drought tolerance QTL mapping had been done. Individual evaluation trials are described in Table 1. Briefly, the non-stress (NS) trials included three rainy season (R), rainfed trials conducted in the same field at Patancheru during 2000, 2001 and 2002 (R2000 NS–R2002 NS), and three fully irrigated sowings during the dry season (D). These latter were used as controls for paired or adjacent terminal stress treatments. The first of these (D2001A NS) was the adjacent, irrigated control planting for three terminal stress environments (D2001A ES, MS and LS)

Table 1

Test environments used in the evaluation of topcross hybrids made with the QTL TCP, Field TCP and Random TCP

Test environment code	Year	Season	Description of test environment	Mean grain yield (g m^{-2})
Non-stress test environments				
R2000 NS	2000	Rainy	Rainfed, non-stress	196
R2001 NS	2001	Rainy	Rainfed, non-stress	385
R2002 NS	2002	Rainy	Rainfed, non-stress	352
D2001A NS	2001	Dry	Fully irrigated	424
D2001B NS	2001	Dry	Fully irrigated	399
D2002 NS	2002	Dry	Fully irrigated	332
Terminal stress test environments				
R2001 ROS	2000	Late rainy	Mild, mid grain filling stress	181
R2001 MS	2000	Late rainy	Mild, mid grain filling stress	154
D2001A ES	2001	Dry	Severe, early grain filling stress	163
D2001A MS	2001	Dry	Severe, mid grain filling stress	205
D2001A LS	2001	Dry	Severe, late grain filling stress	258
D2001B MS	2001	Dry	Moderate, mid grain filling stress	230
D2001B LS	2001	Dry	Moderate, late grain filling stress	351
Gradient stress environments (grain filling)				
D2002 SGE 1	2002	Dry	92% non-stress irrigation	327
D2002 SGE 2	2002	Dry	83% non-stress irrigation	304
D2002 SGE 3	2002	Dry	75% non-stress irrigation	275
D2002 SGE 4	2002	Dry	66% non-stress irrigation	272
D2002 SGE 5	2002	Dry	58% non-stress irrigation	261
D2002 SGE 6	2002	Dry	49% non-stress irrigation	235
D2002 SGE 7	2002	Dry	41% non-stress irrigation	199
D2002 SGE 8	2002	Dry	32% non-stress irrigation	190

The terminal stress environments differed in the time of onset of, and the severity of, the stress (seasonal evaporative demand and plant available soil water). The gradient stress environments differed by the amounts of irrigation water received in weekly irrigations during the grain filling period.

described below. The second (D2001B NS) was the mean of irrigated controls from the two paired stress/non-stress treatments (D2001B MS and LS) also described below. The third control environment (D2002 NS) was the fully irrigated rows, adjacent to the sprinkler line, in the line-source experiment described below.

2.2.2. Terminal stress environments

The terminal stress (S) environments involved a complete termination of irrigation/rainfall. They differed mainly in the time of onset of stress and in the severity of the stress, due to seasonal differences in evaporative demand and the amount of plant-available water in the soil (Table 1). They included two rainy season trials that were sown towards the end of the rains so that that flowering and grain filling would occur after the rains ended, but with artificially extended daylength (Mahalakshmi and Bidinger,

1985) to mimic the normal rainy season photoperiod. One of the two experiments was grown under a rainout shelter to fully exclude rainfall from about 1 week before flowering (R2000A ROS). The other (R2000B MS) was sown in an adjacent field without cover, but did not receive any rains after flowering. Stress in both was relatively mild due to moderate post-rainy season atmospheric vapor pressure deficits and air temperatures. In addition to the rainy season stress environments, there were also five dry season terminal stress treatments that were managed by terminating irrigation at predetermined times. The first three (plus the irrigated control referred to above) were grown in parallel 12 m \times 100 m strips, in which irrigation was terminated at 5 day intervals, beginning with flowering. This created early onset (immediately after flowering = D2001A ES), mid grain filling onset (5–7 days after flowering = D2001A MS) and late grain filling onset (10–12 days after flowering = D2001A

LS) stress environments. Stress was severe in these environments due to high dry season vapor pressure deficits and air temperatures, combined with a soil with relatively low (about 50 mm) plant-available water content. The other two dry season stress environments were the terminal stress treatments of eight-row, paired stress/non-stress plots. A drip irrigation line installed between rows 2 and 3 created the control treatment which was irrigated weekly. The remaining rows were not irrigated after the onset of the stress treatments, creating a paired terminal stress environment in rows 6 and 7. Both stress treatments in this experiment were initiated after flowering, one in the mid grain filling stage (D2001B MS) and the other in the late grain filling stage (D2001B LS). Stress in these treatments was moderate due to the later onset of stress and a soil with greater plant available water than that in the D2001A experiments.

2.2.3. Gradient stress environments

Finally, the entire set of 36 TCH was evaluated under a line-source sprinkler stress gradient during grain filling in the dry season of 2002. In this trial, all test environments received weekly sprinkler irrigation from flowering until maturity, but in decreasing amounts with distance from the line source. Pairs of rows, beginning with rows 4 and 5 (environment D2002 SGE 1, where SGE refers to stress gradient environment) from the sprinkler line, to rows 18 and 19 (environment D2002 SGE 8), were designated as individual stress environments. Water applied to these pairs of rows ranged from 92 to 32% of the water applied to the control rows (Table 1). The stress gradient treatments differed from the terminal stress treatments in that they measured response to varying severity in a set of a common, but partial stress treatments. These were characterized by a variable amount of water available for transpiration each week, or by a variable number of days each week in which there was sufficient water to meet potential evaporation, rather by the timing of onset of an absolute (uninterrupted) stress.

2.3. Field management

All experiments (including the line source) were sown in a split plot design with A-line as main plot and all three TCHs made on that A-line as subplots. This

was done in order to reduce the effects of normal field variation on the comparison of TCPs within individual A-lines. Most trials were sown in two-row \times 5 m long plots (TCH) without border rows between subplots but with border rows (sown to a mixture of the three TCHs on the A-line main plot) between main plots. The only exceptions were R2000 ROS and R2000 MS, which were sown in 1-row plots, and included only 9 of the 12 TCH for each TCP because of limited area under the rainout shelter. Most trials were grown with three replications, except for R2001 NS, R2000 ROS and R2000 MS, which were replicated four times, and D2001B MS and D2001B LS, which were replicated only two times. The line-source experiment was laid out in 20-row wide beds of 5 m width (=1 plot length), at right angles to the line source, with 18 such beds on either side of the line source in each replication. Each bed was sown to one TCH (grouped by A-line); rows 1 (adjacent to the sprinkler line) and 20 were borders. Rows 2 and 3 were considered as the non-stress control, and subsequent pairs of rows from rows 4 and 5 to rows 18 and 19 as stress the gradient environments.

All experiments were machine sown in ridges 0.6 m apart, and stands thinned manually to approximately 0.15 m between plants (11 plants m^{-2}) by 2 weeks after emergence. All trials were well fertilized with 50 kg N and 18 kg P ha^{-1} (as 150 kg ha^{-1} 28-28-0) banded into the ridges before sowing and 23–46 kg N ha^{-1} (as 50–100 kg ha^{-1} urea) side dressed by 15–20 days after emergence. Weed control was by a combination of cultivation and a single hand weeding done just after thinning. Irrigation before the time of initiation of the stress treatments was done by sprinkler in all cases. Sprinkler lines were placed at 12.5 m intervals and sprinklers at 6 m intervals along the line, but with alternate sprinklers run separately to minimize runoff. Each sprinkler line was bordered on each side by two crop rows so that leakage from the lines did not affect experimental rows. The final irrigation in the terminal stress treatments was given by flooding all furrows between the ridges for a 4 h period to completely fill the soil profile. Following the imposition of the stress treatments, the adjacent non-stress control plots were irrigated weekly by flooding alternate furrows. The exception to this was the paired-plot experiments, where the non-stress control plots received a weekly drip irrigation.

The line-source experiment was managed in a similar fashion to the terminal stress treatments, except that following a final furrow irrigation at the heading stage, a line-source sprinkler line was installed in the center of each replication, with sprinklers at 6 m intervals on 2.5 m risers. The sprinkler heads used in conjunction with this arrangement provided a near-linear pattern of water application with distance from the line source. All line-source irrigations were done in the early morning when wind velocity was near zero, to avoid wind distortion of the water application patterns. The line source was operated weekly with the time of application adjusted to approximately replace the previous week's measured pan evaporation in the non-stress rows adjacent to the line. Individual line-source environments were described by the regression-estimated amounts of water applied during the treatment period. These estimates were based on measured amounts of water received in catch cans distributed at right angles to the source in all three replications. Linear coefficients of determination for water applied regressed on distance from the line source ranged from 0.96 to 0.99 ($P < 0.001$) for the individual replications.

2.4. Data collection and analysis

Flowering was recorded in all plots when stigmas were visible on the main shoot panicle of a visually estimated 50% of the plants in the plot. At maturity, panicles were cut from all plants in the center 3 m of both rows (3.6 m²), counted and oven dried at 70 °C for 3 days. Panicles were weighed, mechanically threshed and the grain weighed in a single operation. Duplicate 100 grain samples counted, re-dried and weighed. Following panicle harvest, the stover from the same harvested area was cut at ground level and its fresh weight recorded. A subsample of approximately 1 kg was taken, mechanically chopped, weighed and oven dried at 70 °C for 3 days, and weighed. Stover dry mass was determined from the product of the stover fresh weight and the subsample moisture percentage. Grain yield, dry stover yield and above ground biomass yield (stover dry mass + panicle dry mass) were expressed on a square meter basis. Individual grain mass and grain number per square meter were estimated from the 100 grain samples and the grain yield. Harvest index (grain dry mass/dry biomass) and panicle harvest index (PNHI = grain dry

mass/panicle dry mass) were calculated from the plot yield data. HI provides a general estimate of the success of individual entries in maintaining dry mass allocation to grain yield under post-flowering stress. PNHI provides a specific estimate of the success of individual entries in setting and filling grain under such stress (Bidinger, 2002). This is because a decrease in either grains set or grain filling affects the numerator of the expression to a greater degree than the denominator, which includes the mass of the structural parts of the panicle as well as the grain mass.

Data from individual non-stress and terminal stress trials were analyzed according to the field design by the GLM procedure of SAS, with TCH sums of squares (SS) broken down into A-line, TCP and A-line \times TCP effects. Across-test-environment analyses were done with the same package, with replication nested within environment. TCH \times environment SS broken down into the interactions of environment with A-line, TCP and A-line \times TCP interaction effects. The line-source data were analyzed as single environments (individual SGE) followed by an across environment analysis in the same manner as done for the terminal stress environments. Biomass yield, grain yield and HI were also linearly correlated the amount of water applied to each line-source environment after flowering. Although there were significant A-line effects for all environments/variables and significant (but smaller) A-line \times TCP interactions for many environments/variables (Table 2), yield and yield component data are reported only for TCP means, as the objective was to compare TCPs rather than A-lines or individual TCHs.

Drought response index (DRI) was calculated for all hybrids in the dry season terminal stress environments and the line-source stress environments, according to the procedure of Bidinger et al. (1987b). This procedure estimates drought tolerance/sensitivity independently of the effects of yield potential and drought escape on measured grain yield in a particular stress environment. This allows variation in grain yield under stress to be analyzed in terms of variation in all three factors. Grain yield in each individual stress environment was then modeled as a multiple linear function of yield potential (yield in the irrigated control), drought escape (time to flowering), and drought response (DRI, including zero values), by forward stepwise regression (SAS PROC REGR), using values for all 36 TCHs. Partial

Table 2

Comparison of mean squares for seed parent, topcross pollinator and their interaction for days to flowering, grain yield, biomass, harvest index and panicle harvest index in five sets of test environments (rainy season terminal stress and non-stress, dry season terminal stress and non-stress, and dry season gradient stress)

Source of variation (MS)	d.f.	Days to flowering	Grain yield (g m ⁻²)	Biomass (g m ⁻²)	Harvest index (%)	Panicle HI (%)
Rainy season non-stress						
A-line	11	67.36	14359.0	100904.3	142.08	95.86
Error (main plot)	77	2.77	1564.0	7390.2	7.93	6.19
TCP	2	41.96	23735.7	122020.5	16.49	23.88
A-line × TCP	22	3.71	1416.9	7641.6	4.84	5.67
Error (subplot)	168	1.46	1426.4	5868.5	4.52	5.20
Rainy season stress						
A-line	8	221.54	13221.7	8900.3	529.22	179.68
Error (main plot)	48	8.40	553.8	4712.7	5.63	9.36
TCP	2	150.04	4031.9	9497.0	267.90	52.40
A-line × TCP	16	11.77	609.6	6327.1	11.81	5.71
Error (subplot)	108	7.49	635.3	4232.9	7.29	5.69
Dry season non-stress						
A-line	11	65.09	14372.7	42649.9	224.58	87.36
Error (main plot)	77	1.38	4530.5	14981.1	13.83	4.16
TCP	2	145.43	4429.3	201584.4	387.40	9.37
A-line × TCP	22	3.53	2132.0	6450.5	8.97	5.08
Error (subplot)	168	0.57	923.2	3874.0	4.61	2.29
Dry season stress						
A-line	11	87.18	11651.5	29388.7	455.94	244.85
Error (main plot)	88	1.63	2613.5	8116.1	22.94	24.61
TCP	2	486.87	1607.8	59806.2	513.68	3.36
A-line × TCP	22	4.37	1317.8	7073.1	10.00	6.63
Error (subplot)	192	0.46	640.5	2614.1	7.28	7.00
Dry season line source						
A-line	11	282.15	51677.5	82025.0	1055.77	558.26
Error (main plot)	176	2.15	3042.7	6659.3	26.94	14.90
TCP	2	529.43	25299.3	46746.8	2478.68	384.33
A-line × TCP	22	13.32	5397.4	11625.5	105.95	22.91
Error (subplot)	66	0.82	960.6	2395.2	12.60	7.77

coefficients of determination for each independent variable were considered as an estimate of the relative importance of that variable in determining grain yields in that stress environment. Mean (of all 12 TCHs) values for the partial coefficients of determination for each TCP were used to understand differences among the TCPs in each dry season evaluation environment. MS for TCP mean values for yield potential, drought escape and DRI were tested against the environment × TCP interaction MS.

Finally, grain yield response to selection of the TCP component lines on the basis of either QTL presence or superior performance in the phenotyping experiments was compared by estimating mean % grain

yield advantage of the QTL TCHs and Field TCHs over the TCHs based on the Random TCP. Grain yield advantage in each case was regressed against the trial mean yield for all evaluation environments to assess overall response to selection of TCP component lines across both stress and non-stress environments.

3. Results

3.1. A-line, TCP and interaction effects

Trial environments (Table 1) were grouped according to season (rainy or dry), and treatment

(non-stress, terminal stress and gradient stress) for initial analysis of A-line, TCP and interaction effects. Both A-line and TCP effects for key variables were highly significant across virtually all test environment groups (Table 2). The relative magnitude of the MS of the A-line and TCP effects varied with environment and trait, however. For example, A-line effects for flowering and HI were larger than TCP effects in the rainy season environments, but not in the dry season ones (Table 2). TCP effects were greater than A-line effects for biomass in all but the gradient stress environments, but A-line effects were greater for grain yield in all but the rainy season non-stress environments (Table 2). For the two sets of stress environments, the A-line SS were broken down effects of adaptation group (arid zone versus peninsular India) and of individual A-lines within each adaptation group. In general, A-line effects were due to both between and within A-line adaptation group effects, with a general pattern of the within arid zone group MS > the between group \geq MS peninsular India adapted group MS. For example, in the gradient stress environments the MS for A-line yield differences between the two groups was 10,607 ($P < 0.08$), compared to 93,373 ($P < 0.0001$) within the arid zone adapted group, and 18,101 ($P < 0.0002$) within the peninsular India adapted group (data not presented). A similar analysis for the dry season terminal stress environments gave MS for A-line yield differences between the two groups of 18,213 ($P < 0.001$), compared to 15,235 ($P < 0.0001$) within the arid zone adapted group, and 5690 ($P < 0.07$) within the peninsular India adapted group.

Interactions of A-line and TCP, in contrast, were much smaller than the A-line and TCP effects (Table 2). A-line \times TCP effects in the rainy season environments were generally either non-significant or of borderline significance only. In the dry season environments, both stress and non-stress, A-line \times TCP interactions were generally significant, but at a much lower probability level than the main parental effects. Interaction MS were an order of magnitude (or more) less than parental MS for highly heritable traits such as time to flowering and harvest index and half (or less) of the parental MS in most cases for biomass and grain yield (Table 2). For the two sets of stress environments, the A-line \times TCP SS was also broken down into between adaptation

group \times TCP and within adaptation group \times TCP effects. As in the case of A-line effects, A-line \times TCP effects were also due to both between and within A-line adaptation group \times TCP effects, with a general pattern of the between group \times TCP MS \geq within group \times TCP MS. For example, in the gradient stress environments, the MS for the TCP \times A-line adaptation group interaction for grain yield was 11,587 ($P < 0.0001$), compared to 3693 ($P < 0.001$) for the interaction of TCP \times A-line within the arid zone adapted group, and 5856 ($P < 0.001$) for the interaction of TCP \times A-line within the peninsular India adapted group. In a similar analysis for the dry season terminal stress environments, the MS for TCP \times A-line adaptation group interaction for grain yield was 917 (NS), compared to a MS of 1184 ($P < 0.06$) for the interaction of TCP \times A-line within the arid zone adapted group, and a MS of 1553 ($P < 0.01$) for the interaction of TCP \times A-line within the peninsular India adapted group (data not presented).

3.2. TCH performance in non-stress environments

Mean grain yields in the non-stress evaluation environments were relatively high ($> 3.3 \text{ t ha}^{-1}$), with the exception of the rainy season of 2000 (R2000 NS, Table 1), which was very wet, with the result that crop growth was affected by water logging and normal crop maturation by considerable leaf loss to foliar disease (crop biomass at maturity in this environment was only 5 t ha^{-1} , compared to $8\text{--}9 \text{ t ha}^{-1}$ for the 2001 and 2002 rainy season evaluations). TCP effects (averaged over TCHs and rainy season evaluation environments) were significant for all variables measured (Table 3). The hybrids of both selected TCPs (Field and QTL) had significantly higher HI, individual grain mass and PNHI compared to those made with the Random TCP (Table 3). The Field TCHs were equal or superior to the Random TCHs for all other traits, including grain yield (326 g m^{-2} versus 309 g m^{-2}). In contrast, the QTL TCP hybrids produced a significantly lower above ground biomass (713 g m^{-2}) than either the Field TCP (777 g m^{-2}) or the Random TCP (726 g m^{-2}) in the rainy season non-stress evaluation environments (Table 3). Associated with the lower total biomass in the QTL TCP hybrids was a lower grain number ($30.0 \times 10^3 \text{ m}^{-2}$ versus a mean of $35.0 \times 10^3 \text{ m}^{-2}$ for the Field and Random TCP

Table 3
Summary of comparisons conducted in non-stress environments 2000–2002

Season/year	Days to flowering	Biomass (g m ⁻²)	Harvest index (%)	Grain yield (g m ⁻²)	Grain no. (10 ⁻³ m ⁻²)	Individual grain mass (mg)	Panicle HI (%)
Rainy season							
QTL TCP	38.4	713	41.8	298	30.9	0.973	75.6
Field TCP	39.5	777	41.7	326	34.8	0.942	75.5
Random TCP	39.4	746	41.1	309	35.1	0.884	74.8
<i>P</i> of <i>F</i> _(TCP)	0.0001	0.0001	0.028	0.0001	0.0001	0.0001	0.02
LSD (0.05)	0.31	19.5	0.54	9.6	1.24	0.0209	0.58
Dry season							
QTL TCP	39.1	777	49.5	381	38.8	1.001	80.4
Field TCP	41.3	845	45.9	392	41.7	0.958	79.9
Random TCP	40.7	839	47.1	393	43.0	0.926	79.9
<i>P</i> of <i>F</i> _(TCP)	0.0001	0.0001	0.0001	0.009	0.0001	0.0001	0.02
LSD (0.05)	0.19	15.6	0.55	7.73	1.06	0.018	0.38

Data are means of 12 topcross hybrids made with each topcross pollinator, over three rainy and three dry season trials at Patancheru. See Table 1 for the details of individual trials.

hybrids). The QTL TCHs also flowered 1 day earlier than the Field TCHs or Random TCHs (Table 3). The QTL TCHs did have larger individual grain mass than the hybrids on either of the other two TCPs, which partly compensated for the reduced grain numbers (Table 3). However, the mean grain yield in the QTL TCHs (298 g m⁻²) was still lower than that of either the Field TCHs (326 g m⁻²) or the Random TCHs (309 g m⁻², Table 3).

The general yield component pattern in the dry season non-stress evaluation environments was similar to that in the rainy season non-stress environments, except that the differences between the hybrids made with the two selected TCPs and those with the Random TCP were generally smaller. The Field TCHs again produced marginally more biomass than the Random TCHs (Table 3). However, this did not translate into a larger grain yield, as the HI (41.7%) of the Field TCHs was lower than that of the Random TCHs (43.0%). The lower HI in the Field TCH was likely related to a lower grain number ($41.7 \times 10^3 \text{ m}^{-2}$) compared to the Random TCHs ($43.0 \times 10^3 \text{ m}^{-2}$, Table 3).

The QTL TCHs performed very similarly in the dry season non-stress environments and in the rainy season environments. In comparison to the Random TCP hybrids, they again flowered earlier, produced less biomass (777 g m⁻² versus 839 g m⁻²), and a lower grain number ($38.8 \times 10^3 \text{ m}^{-2}$ versus $43.0 \times 10^3 \text{ m}^{-2}$). As in the rainy season environments, these differences were partly offset by a larger individual grain mass and a higher HI in the QTL

TCHs, but they still yielded significantly less (381 g m⁻²) than the Random TCHs (393 g m⁻², Table 3). Selection on the basis of the putative drought tolerance QTL thus appears to have favored a particular phenotype—earlier flowering, reduced biomass and grain number, but a higher individual grain size, PNHI and HI. This phenotype consistently differed from that resulting from direct selection for the ability to maintain grain yield under stress in the Field TCP.

3.3. TCH performance in terminal stress environments

The terminal stress environments all involved unrelieved (no further water applied) stress treatments. This measured the ability of genotypes to fill grain (and also set grain in the early onset treatments) with whatever carbon assimilation that was possible with the water remaining in the soil and with whatever assimilates were stored in stems. Under such conditions, early flowering (drought escape) is almost always major advantage, particularly for a crop with a short grain-filling period such as pearl millet (Bidinger et al., 1987a; Mahalakshmi et al., 1988).

The same differences in phenotype between the hybrids made with the QTL TCP and those made with the Field and Random TCP noted in the non-stress comparisons were again apparent in both the rainy season and the dry season terminal stress environments. In the milder rainy season terminal stress environments (R2000 ROS and R2000 MS, Table 1),

Table 4

Summary of comparisons conducted in terminal stress and line source gradient stress environments 2000–2002

Season/year	Days to flowering	Biomass (g m ⁻²)	Harvest index (%)	Grain yield (g m ⁻²)	Grain no. (10 ⁻³ m ⁻²)	Individual grain mass (mg)	Panicle HI (%)
Rainy season							
QTL TCP	44.6	549	32.0	175	17.6	0.948	69.2
Field TCP	46.9	572	28.1	160	17.1	0.916	67.7
Random TCP	47.3	559	29.8	166	17.7	0.907	69.2
<i>P</i> of <i>F</i> _(TCP)	0.0001	0.102	0.0001	0.0025	NS	0.0062	0.0002
LSD (0.05)	0.78	18.4	0.77	7.2	–	0.0228	0.68
Dry season							
QTL TCP	41.4	581	41.7	245	31.9	0.769	72.4
Field TCP	43.5	619	38.3	240	33.4	0.716	72.5
Random TCP	43.2	611	38.7	239	34.4	0.689	72.2
<i>P</i> of <i>F</i> _(TCP)	0.0001	0.0001	0.0001	0.084	0.0001	0.0001	NS
LSD (0.05)	0.15	11.4	0.60	5.6	0.83	0.0164	–
Line source							
QTL TCP	35.4	537	49.5	268	34.5	0.795	74.7
Field TCP	38.1	562	43.8	250	34.1	0.749	72.6
Random TCP	36.8	555	45.5	255	36.3	0.719	72.7
<i>P</i> of <i>F</i> _(TCP)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD (0.05)	0.12	7.89	0.51	5.08	0.10	0.014	0.43

Data are means of 9 (rainy season) or 12 (dry season and line source) topcross hybrids made with each topcross pollinator, over 2 rainy and 5 dry season replicated terminal stress trials, and 1 dry season line source trial with 8 stress environments at Patancheru. See Table 1 for the details of individual trials.

the QTL TCHs were earlier flowering and had a higher harvest index and individual grain mass in than either the Field or Random TCP hybrids. But in these environments, they did not have a significantly lower biomass or grain number (Table 4), which offset the advantages of the higher HI and individual grain mass in the non-stress environments (Table 3). As a consequence, the QTL TCHs had a small, but significant ($P < 0.003$) yield advantage in the rainy season stress environments (175 g m⁻² versus an average of 163 g m⁻²) over the Random and the Field TCHs (Table 4). The Field and Random TCHs were similar for most traits measured (Table 4) in the rainy season terminal stress environments, including grain yield (160 g m⁻² versus 166 g m⁻²).

In the more severe dry season stress environments (all D2001 environments in Table 1), the same pattern of differences between the QTL TCH and the other TCH occurred (Table 4). But in these environments, the small grain yield difference between the QTL TCHs (245 g m⁻²) and the Field/Random TCP hybrids (240 g m⁻²) was not significant ($P < 0.084$; Table 4). The Field and Random TCHs were similar for most traits in the dry season stress environments

(except for grain number and individual grain mass, where an advantage in one component was offset by the disadvantage in the other, Table 4). As a consequence, grain yields of the Field and Random TCHs were identical (240 g m⁻² versus 239 g m⁻²). Thus, while the differences in phenotype between the QTL and Field TCHs were consistent across the stress and non-stress environments, the relative grain yields of the two sets of TCHs were not. This suggests a differential adaptation pattern in the Field and QTL TCP phenotypes, and a potential crossover interaction in performance between the stress and non-stress environments.

3.4. TCH performance in gradient stress environments

The gradient stress (line source) evaluation is considered separately because it presents a different challenge to the TCH. In the terminal stress environment, further crop growth, after the onset of the stress, is dependent solely on whatever water remains in the profile. Whereas in the gradient stress the crop receives enough water each week during the

stress period to meet a variable part of its potential transpiration, depending upon its location along the stress gradient, and is thus able to maintain limited growth. The total water applied post-flowering varied from 92 to 32% of the non-stressed control. This resulted in mean yields varying from 327 to 190 g m⁻², compared to a non-stressed control mean yield of 332 g m⁻² (Table 1). While there should still be advantages to early flowering under a gradient stress, it is likely that differences in traits affecting transpiration and assimilation under stress would also be important.

The Field TCHs had a slightly greater response to applied water in terms of biomass produced than Random TCHs (32.7 g m⁻² cm⁻¹ versus 30.0 g m⁻² cm⁻¹, Fig. 1a). However, the difference between the Field and Random TCHs in biomass averaged over the whole gradient was not significant (Table 4). In contrast, HI of the Field TCHs was significantly lower than that of the Random TCHs across the whole gradient (Table 4), due to a significantly smaller intercept value (Fig. 1b). As a consequence, the grain yield responses to applied water of the Field (20.6 g m⁻² cm⁻¹) and Random (20.4 g m⁻² cm⁻¹) TCHs were identical (Fig. 1c). The difference in average grain yields between the Field and Random TCHs across all gradient environments (250 g m⁻² versus 255 g m⁻²) was just at the level of significance (Table 4).

The QTL TCHs produced slightly less biomass across all stress gradient environments than either the Field or Random TCHs (Fig. 1a). Neither the intercepts nor the slopes of the regressions of biomass on water applied were significantly different between the QTL TCHs and Random TCHs (data not presented). However, the combined differences resulted in a significant difference in biomass between the QTL and Random TCHs when averaged over the whole set of gradient stress environments (Table 4). As was the case in other environments, the QTL TCHs maintained a significantly greater partitioning of biomass to grain across the whole water gradient than did the Random or the Field TCHs (Fig. 1b). This was due to differences in intercept (HI at zero additional water applied) where the HI for the QTL TCHs was 42.2% versus 37.3% for the Random TCHs and 36.2% for the Field TCHs (Fig. 1b). Averaged over the whole gradient, differences in HI between the

QTL TCHs and the other groups of hybrids were highly significant (Table 4). As a consequence of the differences in HI, the QTL TCHs maintained a significant grain yield advantage over the entire gradient (Fig. 1c, Table 4). This advantage in grain yield was due to a greater regression intercept, similar to the case of HI (135 g m² for the QTL TCHs versus 112 g m⁻² for the Field TCHs and 118 g m⁻² for the Random TCHs). These differences in the QTL TCHs and the Field/Random TCHs are consistent with earlier observations from both the rainy season and dry season terminal stress environments (Table 4).

The other variable for which the QTL TCHs exceeded the other two groups in the gradient stress environments was PNHI (Table 4). As in the case of HI, this was due to a larger intercept value—the QTL TCHs had a mean 67.7% PNHI at zero applied water, compared to an average of 65.5% for the other two groups of hybrids (Fig. 1d). This may be indicative of a superior ability to fill grain under the specific conditions of the gradient stress, and is consistent with the larger average grain size in the QTL TCHs across all gradient environments (Table 4). However, this superior PNHI in the QTL TCHs was not evident in the terminal stress environments (Table 4), and while present (and significant) in the non-stress environments, was very small (Table 3).

4. Discussion

4.1. Differences in TCP performance in stress environments

We analyzed the TCH (and thereby TCP) differences in grain yield under terminal stress in terms of effects of yield potential, drought escape and response to drought. In the terminal stress environments, variation in drought escape and drought response (represented by DRI) each accounted for approximately 37% of the variation in grain yield among the 36 TCHs in the trials, compared to 22% for variation in yield potential (Table 5). Thus, differences among the three groups of TCH in any one or more than one of these three components could explain the observed yield differences in the terminal stress environments. The QTL TCHs were on average significantly earlier flowering than both the Field

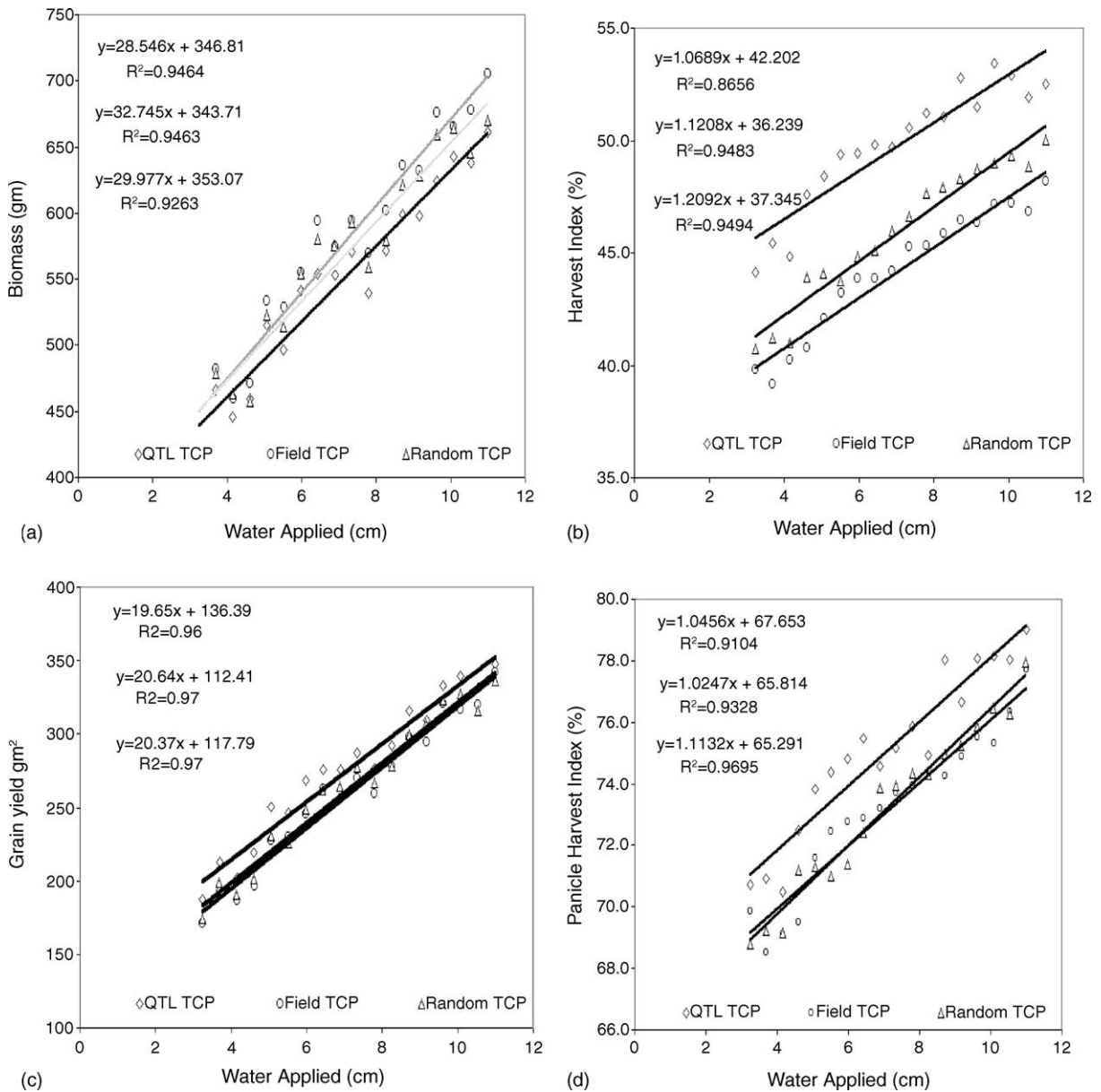


Fig. 1. Comparison of the response of QTL TCP hybrids (\diamond), Field TCP hybrids (\circ) and Random TCP (\triangle) to the amount of irrigation water applied after flowering in the gradient stress environments in terms of: (a) biomass; (b) harvest index; (c) grain yield; and (d) panicle harvest index.

and Random TCHs, which undoubtedly contributed to their higher yield across all the terminal stress environments (in view of the proportion of the variation in grain accounted for by drought escape, Table 5). In contrast, the QTL TCHs had a significantly lower mean yield potential in the terminal stress/non-

stress comparisons than the Field and Random TCHs (Table 5). Although yield potential was a lesser factor in determining grain yield under terminal stress than either escape or DRI, the grain yield of the QTL TCHs under stress was still likely affected to some degree by their lower yield potential. Although DRI was equally

Table 5

Yield potential, drought escape, drought tolerance/susceptibility (DRI) and yield in the stress environments of QTL, Field and Random TCHs, across dry season terminal stress/non-stress environments, line source gradient stress environments, across all dry season stress environments

Effect conditioning grain yield and mean stress environment yield	QTL TCP hybrids	Field TCP hybrids	Random TCP hybrids	Probability of difference ^a	LSD (0.05)
Across terminal stress environments					
Yield potential (g m^{-2}) (pCD = 0.220)	403	425	428	0.0001	7.2
Time to flowering (d) (pCD = 0.363)	41.6	43.7	43.3	0.0001	0.21
Drought response (pCD = 0.368)	−0.044	+0.206	−0.176	0.247	–
Yield in stress (g m^{-2})	245	240	239	0.033	6.7
Across gradient stress environments					
Yield potential (g m^{-2}) (pCD = 0.792)	339	331	326	0.13	–
Time to flowering (d) (pCD = 0.003)	35.5	38.0	36.9	0.0001	0.43
Drought response (pCD = 0.198)	+0.294	−0.425	+0.206	0.0002	0.37
Yield in stress (g m^{-2})	261	242	247	0.0001	4.3
Across all stress environments					
Yield potential (g m^{-2}) (pCD = 0.543)	375	383	382	0.0005	4.9
Time to flowering (d) (pCD = 0.209)	38.9	41.2	40.5	0.0001	0.15
Drought response (pCD = 0.204)	+0.107	−0.074	−0.006	0.50	–
Yield in stress (g m^{-2})	252	241	243	0.0001	4.6

The pCD for each effect is the partial coefficient of determination from forward stepwise regressions of grain yield under stress on yield potential, drought escape, and drought tolerance/susceptibility, using data from all 36 TCH.

^a The probability of the difference among TCP is based on the ratio of the MS for TCP and the MS for the interaction on TCP \times A-line \times environment in the analysis of variance model.

important as drought escape in determining individual TCH grain yields under stress, there were no statistical differences in mean DRI among all three groups of TCHs (Table 5). Therefore, differences in drought tolerance/susceptibility (as defined here) did not contribute to yield differences among groups. It thus appears that the marginal yield superiority ($P < 0.04$) of the QTL TCP hybrids, as a group, in the terminal stress environments was due primarily to their generally earlier flowering.

The same analysis for the gradient stress environments provided a rather different picture. Here, variation for grain yield potential accounted for nearly 80% of the variation in actual yields among the individual TCHs (Table 5). The remaining 20% of the variation in yield under stress among the TCHs was accounted for by variation in DRI; variation in time to flowering made little contribution to yield under stress. Despite the existence of differences in yield potential among individual TCH, differences in the mean yield potential among the three groups of TCH were not significant ($P = 0.13$) suggesting that the yield superiority of the QTL TCHs as a group in the gradient stress environments was not due to differences in potential yield (Table 5). Differences in DRI

among the three groups of TCHs, however, were highly significant ($P < 0.0002$), and in the same order (QTL > Random > Field) as the differences in measured grain yield (Table 5). Thus, in the gradient stress environments (in contrast to the terminal stress environments) the yield superiority of the QTL TCHs, as a group, is likely due to a greater tolerance to the gradient stress, rather than to drought escape.

The fact that the gradient stress environment differed from the terminal stress ones in that it did allow continued plant growth after the onset of the stress. This raises the question of whether differences in stress tolerance/susceptibility were related to differences in crop growth, which might indicate how tolerance/susceptibility to the gradient stress was expressed (Bidinger et al., 1987b; Fussell et al., 1991). Unfortunately, DRI in the gradient stress environments was not strongly correlated to any of the measured crop/yield parameters. The strongest correlation of DRI (across all TCHs) was to grain yield per panicle ($r = 0.41$, $P < 0.01$) but DRI was not uniquely correlated to either component of grain yield per panicle—grain number per panicle ($r = 0.33$, $P < 0.05$) or individual grain mass ($r = 0.24$, $P > 0.10$). However, the QTL TCHs had both a larger

individual grain size ($0.80 \text{ mg grain}^{-1}$) and numerically greater grain number per panicle (1378) than both the Field TCH ($0.75 \text{ mg grain}^{-1}$ and 1341 grains panicle^{-1}) and the Random TCH ($0.72 \text{ mg grain}^{-1}$ and 1355 grains panicle^{-1}). Both components likely contributed to the greater DRI of the QTL TCHs as a group. Whether or not this was the entire difference is not known.

Averaged over both the terminal stress and the gradient stress environments, the QTL TCHs had a modest but highly significant ($P < 0.0001$) yield advantage over the Field and Random TCHs (Table 5). Across all the stress environments, grain yield potential accounted for 54% of the variation in individual TCH yield, compared to 21% for time to flowering and 20% for DRI (Table 5). The mean potential yield of QTL TCHs were significantly ($P < 0.0005$) less than that of the other two groups of hybrids, but the QTL TCH were significantly ($P < 0.0001$) earlier flowering (Table 5). DRI averaged over both terminal stress and gradient stress environments did not differ among the three groups of hybrids, even though this appeared to be the major reason for the QTL TCH advantage in the gradient stress environments. It is possible that the drought tolerance of QTL TCH is better (or only) expressed in environments in which they are able to maintain some level of leaf gas exchange. Thus, the only consistent advantage of the QTL TCH over the Field and Random TCH across all stress environments was earlier flowering (Table 5). The absolute difference in mean time to flowering across all stress environments between the QTL TCHs and the Field and Random TCHs was small (2 days), but in percentage terms was similar to the mean yield advantage of the QTL TCP hybrids: +4.8% for time to flowering and +4.1% for grain yield (Table 5). Whether or not this small difference in flowering is sufficient to explain the measured differences in grain yield is not clear, but it represents the only statistical difference among the groups of TCH across all stress evaluation environments.

4.2. TCH phenotype and terminal drought tolerance

The small superiority of the QTL TCHs in grain yield under stress in both the terminal and gradient stress environments (whether due to drought escape or

drought tolerance) may be more broadly related to the particular phenotype of the QTL TCHs. Besides being earlier flowering, the QTL TCHs produced less biomass and a lower total grain number, but had a larger individual grain mass and a higher HI in all environments, plus a higher PNHI under terminal stress, compared to the other two groups of TCH (Tables 2 and 3). This closely reflects the phenotype of the tolerant parent (PRTL 2/89-33) of the mapping population. It also appears to be a consequence of the selection of the progenies used to make this TCP on the basis of the PRTL 2/89-33 allele at the LG 2 drought tolerance QTL. QTL analysis of the original mapping population progenies grown in non-stress environments identified QTL on LG 2 for all these traits, for which the PRTL 2/89-33 allele was associated with lower values (Yadav et al., 2003). This would therefore predict that the QTL TCP would have a fewer panicles and lower biomass, stover yield and grain yield. Similarly, there is a secondary QTL for flowering time on LG 2, with the PRTL2/89-33 allele associated with earlier flowering under terminal stress (Yadav et al., 2002). The major flowering time QTL were found on linkage groups 4 and 6 (Yadav et al., 2002.). Thus, it is likely that the phenotype of the QTL TCHs was “selected” in the process of the choosing progenies with the LG 2 drought tolerance QTL. Interestingly, however, this phenotype was not associated with the progenies with superior grain yield under terminal stress selected to make the Field TCP.

This phenotype is generally associated with the *Iniadi* landrace (Andrews and Anand Kumar, 1996), from which PRTL 2/89-33 was derived and which is generally adapted to terminal stress environments (authors, unpublished data). The early flowering character of this phenotype clearly allows partial escape from an end-of-season drought stress (Mahalakshmi et al., 1988); even a few days difference in flowering can have an effect in a crop with a grain filling period of approximately 20 days (Bidinger and Hash, 2003). The lower grain number/larger grain size phenotype of the *Iniadi* landrace is associated with reduced effective basal tillering and a larger proportion of the crop yield accumulated in the main shoot panicle (Bidinger and Raju, 2000). Compared to higher tillering/smaller panicle phenotypes, this will limit the percentage yield reduction due to the terminal stress-induced loss of later-developing tillers (van

Oosterom et al., 2003). The normally high HI of the *Iniadi* phenotype is also an indication of efficient partitioning of biomass to reproductive structures. Efficient partitioning should provide an advantage under conditions of limited assimilation due to drought stress, as in the gradient stress environments. Finally, a higher PNHI under terminal stress is associated with a higher percentage of grain set and/or a greater degree of grain filling under limited assimilate conditions (Bidinger, 2002). Interestingly, the superiority of the QTL TCHs in terms of PNHI was most marked in the gradient stress environments, where the partial maintenance of leaf gas exchange during the whole grain filling period should have provided a better opportunity (compared to the terminal stress environments) for the expression of mechanisms improving grain filling under stress.

This putative drought tolerant phenotype has no advantage in favorable, full season environments, however, as early flowering limits total biomass accumulation, and reduced effective tillering may limit radiation interception (Bidinger and Hash, 2003). These effects were evident in the non-stress environments in both seasons, where the QTL TCHs yielded significantly less than the Field or Random TCHs, due primarily to lower biomass production and lower grain number (Table 3). The non-stress environment of the line-source was the only environment in which the QTL TCHs yielded as much as the Field and Random TCHs in the absence of stress. This was likely due to the inherently high harvest index of the QTL TCHs in an environment in which biomass accumulation was limited in all genotypes by the very short time to flowering (<40 days, due to the combination of short day lengths in February and above average temperatures in 2002).

4.3. LG 2 drought tolerant QTL as a selection criterion

In the final analysis, the real value of a putative drought tolerance (or any) QTL is its effectiveness as a selection criterion. This means that it should not only produce cultivars that are superior to the base population from which selection was done, but also cultivars that are superior to those produced by alternative selection criteria. This superiority should be most evident in the environments specifically

targeted by the selection, and, in general, should not be at the cost of performance in other environments that are a part of the breeding program's overall target population of environments. Many plant breeders would be reluctant to accept an improved grain yield in a drought stress target environment if this were at the cost of a significant yield disadvantage in non-stress environments.

There was a consistent, if modest, grain yield advantage in the terminal stress environments in response to selection of TCP component lines on the basis the putative drought tolerance QTL (Table 4; Fig. 1), but this was clearly at the cost of TCH yield in both the rainy and dry season non-stress environments (Table 3). To explore this apparent crossover a bit further, the yield advantage of the QTL TCH over the Random TCH (representing the yield of the original population) was plotted against the environmental mean grain yield for each trial environment. The results clearly demonstrate that the response to selection for the PRLT2/89-33 allele LG 2 putative drought QTL is a function of the mean grain yield of the test environment, with a negative yield response to selection in high yielding environments and a positive response in lower yielding ones (Fig. 2a). The fitted regression predicts that where mean environmental yield is above 327 g m^{-2} , the response to selection (advantage over the original population mean) will be negative, and vice-versa. Response to selection on the basis of the LG 2 drought QTL will be agronomically useful ($\geq 5\%$ yield gain) only below an environmental mean yield of 227 g m^{-2} . As discussed above, this appeared to be due to the fact that selection based on the presence of the LG 2 QTL appeared to favor a specific plant type adapted to terminal drought stress. However, this plant type is unable to fully exploit high yield potential environments, so selection based on this plant type carries a penalty in terms of yield potential. The original mapping population progenies used to make the QTL TCP were selected only on the basis of PRLT2/89-33 allele at the LG 2 QTL; no assessment was made of the parental alleles at other loci. Thus, these lines could have had a high proportion of PRLT2/89-33 alleles at linked loci on LG 2 (or other linkage groups), resulting in the phenotypic similarity of the QTL TCP and the drought tolerant parent of the original population.

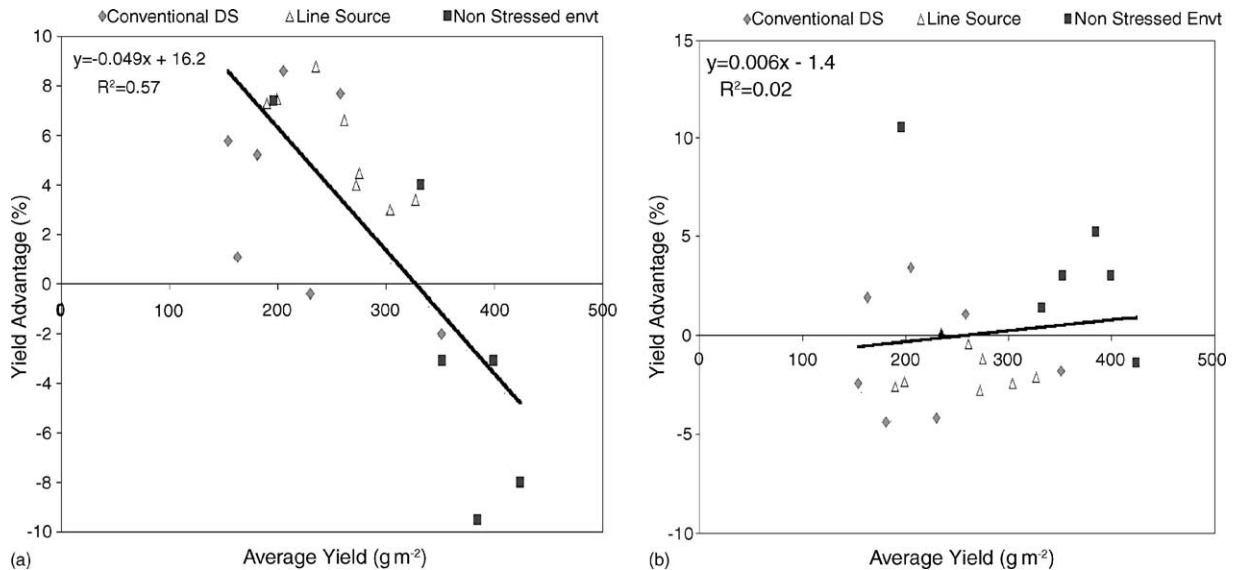


Fig. 2. Percent grain yield advantage of: (a) the QTL TCP hybrids and (b) the Field TCP hybrids over Random TCP hybrids as a function of the mean yield of individual non-stress (□), terminal stress (◇) and gradient stress (△) evaluation environments.

The advantage of the Field TCHs over the Random TCHs, in contrast, was uniformly negligible, as likely to be negative as positive, and unrelated to the yield level or the moisture availability of the evaluation environment (Fig. 2b). Thus, direct selection of the component lines of the Field TCP for the ability to maintain grain yield in the terminal drought stress environments used in the phenotyping of the population (Yadav et al., 2002) was clearly ineffective. The phenotype of the Field TCHs resembles that of the majority of the original mapping population lines, as represented by the Random TCHs, rather than the phenotype of the QTL TCHs (Tables 2 and 3). The lower (compared to the QTL TCHs) grain yields of the Field TCHs in the stress environments are therefore consistent with the apparent linkage of the plant type of the drought tolerant parent PRLT 2/89-33 and the QTL TCHs with adaptation to terminal stress environments. Linkage or co-mapping of QTL for grain yield under stress and QTL tolerance mechanisms/phenotypic traits has been reported in other cereals (e.g. in maize: Agrama and Moussa, 1996; Ribaut et al., 1997). The obvious interpretation is that the linked or co-mapped QTL represent traits or mechanisms that are sufficiently important in main-

taining yield under stress, that they appear as QTL for yield as well. This seems to be the case with the association of phenotype and yield under stress reported here.

If adaptation (measured as yield advantage) to terminal stress in pearl millet is most easily achieved by selection for a specific phenotype, rather than for more basic physiological or biochemical tolerance mechanisms or responses, then the breeder is likely to be facing the tradeoff represented by the QTL TCHs. Effectively therefore, the breeder will be faced with the need to breed for specific adaptation to terminal stress prone environments, rather than breeding for wider adaptation to both stress and non-stress environments. This case has been made where stress environments are very low yielding and/or require very different adaptive mechanisms that non-stress ones (Ceccarelli, 1994; Simmonds, 1991). The identification of pearl millet with environments in which drought stress is a regular feature (Bidinger and Hash, 2003) makes this a very likely scenario.

The resolution of this dilemma depends upon whether the association of better performance in terminal stress environments with the phenotype represented by PRLT 2/89-33 and the QTL TCP is

causal (pleiotropic) or not. If this association of plant type and terminal stress tolerance is causal, marker-assisted backcrossing to improve drought tolerance using this QTL is likely to have difficulties in both improving adaptation to terminal stress and recovering the recurrent parent phenotype and its yield potential. If the association of plant type and terminal stress tolerance is not causal (linkage), it should be possible to transfer improved drought tolerance (associated with the PRLT 2/89-33 allele at the LG 2 drought tolerance QTL) to any recurrent parent phenotype, without a penalty in yield potential. Even if the association is not causal, the success of such a marker-assisted backcrossing program will largely depend upon the size of the genomic region incorporated, and the effects of donor parent alleles at adjacent loci. If the genomic region containing the QTL is large there is a high probability that it also contains a large number of linked donor parent alleles. This implies a similar probability that transfer of this region into another genetic background would result in the transfer of at least some components of the trait complex that together resulted in the drought tolerant plant type in this experiment. Therefore, the associations between plant type and yield performance in various moisture regimes seen in this experiment would also likely occur in the backcross-derived materials. If the genomic region responsible for this drought tolerance QTL were small, it would be easier to break undesirable linkages with other donor parent alleles. This would make it possible to separate QTL effects from plant type effects and avoid the yield penalty associated with the donor parent phenotype.

A number of marker-assisted backcross versions of the drought sensitive parent, H 77/833-2, into which the allele(s) from PRLT 2/89-33 at the LG 2 drought tolerance QTL have been introgressed (see Hash et al., 2000) are now completing field evaluation. The original high tillering, small-panicle H 77/833-2 (drought sensitive) phenotype has been largely recovered in at least some of the backcross progeny. Initial evaluation results indicate that it has been possible to improve grain yield under terminal stress in these lines without a biomass penalty under stress conditions or the grain yield penalty in well-watered conditions (Serraj et al., *in press*; Hash et al., 2004). Thus, it is likely that the association of drought

tolerance and plant phenotype in this experiment is a consequence of linkage rather than pleiotropy.

5. Conclusions

The evaluation of the QTL, Field and Random TCHs confirmed the value of the PRLT2/89-33 allele at the putative drought tolerant QTL on LG 2. The QTL TCHs were consistently and significantly higher yielding in both the unrelieved terminal stress and the gradient stress evaluation environments, than were both the Field or Random TCHs. This advantage under stress was at the cost of a lower yield in the non-stress environments, however. The QTL TCH had a phenotype similar to that of the drought tolerant mapping population parent PRLT-2/89-33. This particular phenotype—early flowering, reduced basal tillering and a highly productive main shoot panicle—appeared to be consistent with the observed differences in the performance of the QTL-based hybrids in both the stress and non-stress evaluation environments. These results raise the question of whether the putative drought tolerance QTL on LG 2 in pearl millet (as selected in this experiment) influences adaptation to terminal drought stress by selecting for an adaptive phenotype or by affecting crop response to drought stress as a more basic biochemical or physiological level.

Acknowledgments

This document is an output from a project (Plant Sciences Research Programme R7375) funded by the UK Department for International Development (DFID) and administered by the Centre for Arid Zone Studies (CAZS) for the benefit of developing countries. The views expressed are not necessarily those of DFID. Special thanks to Mr. D. Dharani, Mr. P.V.D. Maheshwar Rao and Mr. N. Pentaiah for producing the seed for and managing the data collection from the field trials reported here, and to Ms. S.V.L.S.S. Katyayani Devi for assistance in data analysis. The RFLP genotyping of mapping population progenies, performed by Dr. Graeme P. Cavan at IGER (during an earlier DFID-supported project), from which the component parental lines of the three

pearl millet topcross pollinators reported in this study were bred, is also gratefully acknowledged.

References

- Agrama, H.A., Moussa, M.E., 1996. Mapping QTLs in breeding for drought tolerance in maize (*Zea mays* L.). *Euphytica* 91, 89–97.
- Allouis, S., Qi, X., Lindup, S., Gale, M.D., Devos, K.M., 2001. Construction of a BAC library of pearl millet, *Pennisetum glaucum*. *Theor. Appl. Genet.* 102, 1200–1205.
- Andrews, D.J., Anand Kumar, K., 1996. Use of the West African pearl millet landrace *Iniadi* in cultivar development. *Plant Genet. Res. Newsl.* 105, 15–22.
- Bidinger, F.R., 2002. Field screening for drought tolerance: principles and illustrations. In: Saxena, N.P., O'Toole, J.C. (Eds.), *Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice*. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, pp. 109–124.
- Bidinger, F.R., Hash, C.T., 2003. Pearl millet. In: Nguyen, H.T., Blum, A. (Eds.), *Physiology and Biotechnology Integration for Plant Breeding*. Marcel Dekker, New York, pp. 225–270.
- Bidinger, F.R., Mahalakshmi, V., Rao, G.D.P., 1987a. Assessment of drought resistance in pearl millet (*Pennisetum americanum* (L.) Leeke). I. Factors affecting yields under stress. *Aust. J. Agric. Res.* 38, 37–48.
- Bidinger, F.R., Mahalakshmi, V., Rao, G.D.P., 1987b. Assessment of drought resistance in pearl millet (*Pennisetum americanum* (L.) Leeke). II. Estimation of genotype response to stress. *Aust. J. Agric. Res.* 38, 49–59.
- Bidinger, F.R., Raju, D.S., 2000. Mechanisms of adjustment by different pearl millet plant types to varying plant population densities. *J. Agric. Sci. (Camb.)* 134, 181–189.
- Breese, W.A., Hash, C.T., Devos, K.M., Howarth, C.J., 2002. Pearl millet genomics: an overview with respect to breeding for resistance to downy mildew. In: Leslie, J.F. (Ed.), *Sorghum and Millets Pathology*. Iowa State University Press, Ames, Iowa, USA, pp. 243–246.
- Budak, H., Pedraza, F., Cregan, P.B., Baenziger, P.S., Dweikat, I., 2003. Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. *Crop Sci.* 44, 2284–2290.
- Ceccarelli, S., 1994. Specific adaptation and breeding for marginal conditions. *Euphytica* 77, 205–219.
- Eldin, M., 1993. Analyse de l'effet des deficits hydriques sur la recolte du mil au Niger, consequences agronomiques. In: Hamon, S. (Ed.), *Le Mil en Afrique, Diversite Genetique et Agro-physiologique: Potentialites et Contraintes pour l'Amelioration et la Culture*. ORSTOM, Paris, pp. 149–160.
- Fussell, L.K., Bidinger, F.R., Bieler, P., 1991. Crop physiology and breeding for drought tolerance: research and development. *Field Crops Res.* 27, 183–199.
- Hash, C.T., Rizvi, S.M.H., Serraj, R., Bidinger, F.R., Vadez, V., Sharma, A., Howarth, C.J., Yadav, R.S., 2004. Field assessment of backcross-derived hybrids validates a major pearl millet drought tolerance QTL. *Agronomy Abstracts*.
- Hash, C.T., Yadav, R.S., Cavan, G.P., Howarth, C.J., Liu, H., Qi, X., Sharma, A., Kolesnikova-Allen, M.A., Bidinger, F.R., Witcombe, J.R., 2000. Marker-assisted backcrossing to improve drought tolerance in pearl millet. In: Ribaut, J.-M., Poland, D. (Eds.), *Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited Environments. A Strategic Planning Workshop held at International Maize and Wheat Improvement Center (CIMMYT), El Batan, Mexico, D.F., Mexico, 21–25 June 1999*, pp. 114–119.
- Kapoor, R.L., Karkar, P.S., Khairwal, I.S., Bainiwal, C.R., Nijhawan, D.C., Yadav, H.P., 1989. Bajra hybrid HHB 67: a major break through. *Haryana Farming* 18, 17–21.
- Ludlow, M.M., Muchow, R.C., 1990. A critical review of traits for improving crop yield in water-limited environments. *Adv. Agron.* 43, 107–153.
- Mahalakshmi, V., Bidinger, F.R., 1985. Water stress and time of floral initiation in pearl millet. *J. Agric. Sci. (Camb.)* 105, 437–445.
- Mahalakshmi, V., Bidinger, F.R., Raju, D.S., 1988. Timing and intensity of water deficits during flowering and grain filling in pearl millet. *Agron. J.* 80, 30–135.
- Mahalakshmi, V., Bidinger, F.R., Raju, D.S., 1987. Effect of timing of water stress on pearl millet (*Pennisetum americanum*). *Field Crops Res.* 15, 327–339.
- Nguyen, H.T., Chandra Babu, R., Blum, A., 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. *Crop Sci.* 37, 1426–1434.
- Prioul, J.-L., Quarrie, S., Causse, M., de Vienne, D., 1997. Dissecting complex physiological functions through the use of molecular quantitative genetics. *J. Exp. Bot.* 48, 1151–1163.
- Qi, X., Lindup, S., Pittaway, T.S., Allouis, S., Gale, M.D., Devos, K.M., 2001. Development of simple sequence repeat markers from bacterial artificial chromosomes without subcloning. *Bio-techniques* 31, 355–362.
- Qi, X., Pittaway, T.S., Lindup, S., Liu, H., Waterman, E., Padi, F.K., Hash, C.T., Zhu, J., Gale, M.D., Devos, K.M., 2004. An integrated genetic map and a new set of SSR markers for pearl millet, *Pennisetum glaucum*. *Theor. Appl. Genet.* 190, 1485–1493.
- Quarrie, S.A., Stojanovic, J., Pekic, S., 1999. Improving drought resistance in small-grained cereals: a case study, progress and prospects. *Plant Growth Reg.* 29, 1–21.
- Ribaut, J.-M., Betran, J., 1999. Single large-scale marker-assisted selection (SLS-MAS). *Mol. Breed.* 21, 1–11.
- Ribaut, J.-M., Jiang, C., Gonzalez-de-Leon, D., Edmeades, G.O., Hoisington, D.A., 1997. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. *Theor. Appl. Genet.* 94, 887–896.
- Richards, R.A., 1996. Defining selection criteria to improve yield under drought. *Plant Growth Reg.* 20, 157–166.
- Schneider, K.A., Brothers, M.E., Kelly, J.D., 1997. Marker-assisted selection to improve drought resistance in common bean. *Crop Sci.* 37, 51–60.
- Serraj, R., Hash, C.T., Rizvi, S.M.H., Sharma, A., Yadav, R.S., Bidinger, F.R. Recent advances in marker-assisted selection for drought tolerance in pearl millet. *Plant Prod. Sci.*, in press.

- Simmonds, N.W., 1991. Selection for local adaptation in a plant breeding programme. *Theor. Appl. Genet.* 82, 363–367.
- Stuber, C.W., Polacco, M., Senior, L., 1999. Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. *Crop Sci.* 39, 1571–1583.
- Turner, N.C., Wright, G.C., Siddique, K.H.M., 2000. Adaptation of grain legumes (pulses) to water-limited environments. *Adv. Agron.* 71, 193–231.
- van Oosterom, E.J., Bidinger, F.R., Weltzien, R.E., 2003. A yield architecture framework to explain adaptation of pearl millet to environmental stress. *Field Crops Res.* 80, 33–56.
- van Oosterom, E.J., Whitaker, M.L., Weltzien, R.E., 1996. Integrating genotype by environment interaction analysis, characterization of drought patterns, and farmer preferences to identify adaptive plant traits in pearl millet. In: Cooper, M., Hammer, G.L. (Eds.), *Plant Adaptation and Crop Improvement*. CAB International, Wallingford, UK, pp. 383–402.
- Yadav, R.S., Hash, C.T., Bidinger, F.R., Cavan, G.P., Howarth, C.J., 2002. Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought stress conditions. *Theor. Appl. Genet.* 104, 67–83.
- Yadav, R.S., Bidinger, F.R., Hash, C.T., Yadav, Y.P., Yadav, O.P., Bhatnagar, S.K., Howarth, C.J., 2003. Mapping and characterisation of QTL \times E interactions for traits determining grain and stover yield in pearl millet. *Theor. Appl. Genet.* 106, 512–520.
- Yadav, R.S., Hash, C.T., Bidinger, F.R., Devos, K.M., Howarth, C.J., 2004. Genomic regions associated with grain yield and aspects of post-flowering drought tolerance in pearl millet across stress environments and testers background. *Euphytica* 136, 265–277.